PARTICULATE MATTER

Airway Effects of Airborne Environmental Agents

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Environmental Issue

For reasons that are poorly understood, asthmatics appear to be particularly sensitive to the effects of certain air pollutants — including ozone (O_3) an oxidant gaseous pollutant and particulate matter.

Asthma, an inflammatory airways disease, has become an urgent health problem affecting an estimated 17 million persons in the United States alone (CDC 1998 MMWR 47). Since 1979, the death rate from asthma has increased by almost 56%.

Furthermore, elderly asthmatics are experiencing some of the highest mortality rates of any age group. Data are needed to better understand why individuals with asthma or other types of lung disease appear to be at increased risk for air pollutant exposure.

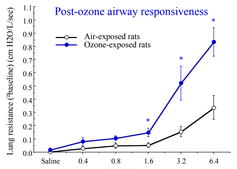
Object/Study Goal

The general purpose of these studies was to expand and improve upon toxicologic testing methods to better assess mechanisms by which air pollution exposure affects the airways.

We have used a combination of *in vivo* and *in vitro* approaches to investigate whether airway epithelial injury and cellular oxidative stress play a critical role in the linkages between air pollutant exposure and development of adverse respiratory health effects or exacerbation of inflammatory airways disease.

ORD Air Sub-Objective - Tropospheric Ozone Research: Advancements Made to Study Effects of Ozone on Susceptible Populations.

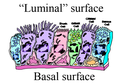
Results Summary — In collaboration with colleagues in the Human Studies Division, we developed a variety of methods to assess ozone-induced respiratory effects. For example, we developed an inhalation bronchoprovocation testing procedure that detected increased airway responsiveness in geriatric rats exposed to ozone. Increased airway responsiveness is a hall-mark feature of asthma in humans. We also determined that certain rat strains are more susceptible to ozone-induced effects including development of airway inflammation, another key feature of asthma. Furthermore, using a rodent airway epithelial cell culture system, we exposed epithelial cells directly to ozone and observed that multiple inflammatory mediator pathways were affected. Collectively, our results suggest that certain individuals, possibly those with pre-existing airway disease (such as asthmatics), are likely to be more susceptible to the effects of oxidant pollutants.

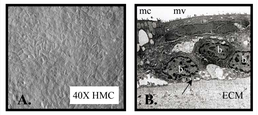


Bronchoprovocation testing in 14-mo-old F344 rats demonstrated that nonspecific airway responsiveness increased significantly 2 h after a 2 ppm x 2 h ozone exposure.

In vitro airway model

Rat-derived primary airway epithelial cell cultures were established at an air-liquid interface.

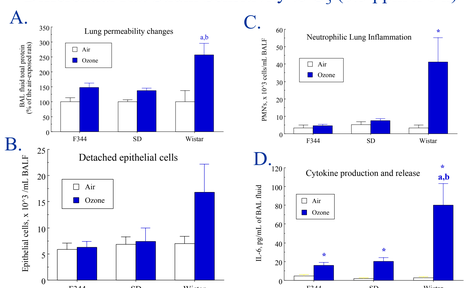




Inverted microscopic (A) and transmission electron microscopic (B) views reveal that a "field" of tightly adherent, pseudodifferentiated muco-ciliary epithelial cells have become established.

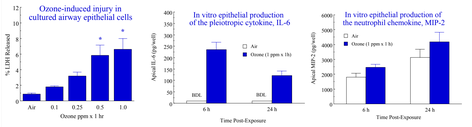
Analogous to the *in vivo* state, these epithelial cultures contain a variety of cell types including mucus-producing (mc) cells, basal (b) cells, and cells with cilia or numerous microvilli (mv). Also present are cell-to-basement membrane (arrow) contacts and a modified subjacent extracellular matrix (ECM) layer .

Differential Rat Strain Sensitivity to O_3 (0.5 ppm x 8 h)



Ozone-induced acute (A) lung and (B) epithelial injury, as well as (C) airway inflammation and (D) inflammatory mediator release were consistently greater in Wistar rats.

Ozone-induced effects in Wistar-derived airway epithelial cell cultures.



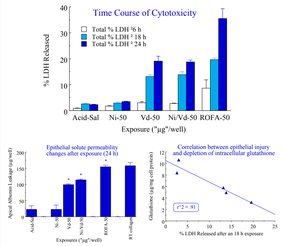
Associated publication

- Dye JA, Madden MC, Richards JH, Lehmann JR, Devlin RB, Costa DL. Ozone effects on airway responsiveness, lung injury, and inflammation. Comparative rat strain and *in vivo/in vitro* investigations. *Inhalation Toxicol*, 1999; 11:1015-1040.
- This paper was awarded honorable mention for its comprehensive and critical evaluation of the parallelisms between *in vivo* and *in vitro* investigations on ozone-induced pulmonary health effects by the EPA Scientific and Technological Achievement Award (STAA) program(2000).

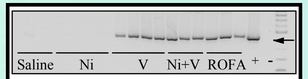
ORD Air Sub-Objective - Particulate Matter Research Health Risk: Advancements Made to Study Effects of PM on Susceptible Populations.

Results Summary — Our investigations described PM characteristics of a fugitive residual oil fly ash that were most directly associated with biologic responses induced in the exposed airway. Using primary airway epithelial cultures derived from Sprague-Dawley rats, we compared and contrasted the acute effects of an intact fly ash sample with that of its principal constitutive transition metals, namely iron, nickel, and vanadium. In brief, vanadium— seemingly via oxidative stress-mediated mechanisms — appeared to be driving the majority of the airway epithelial effects induced by this emission source PM sample. Qualitatively, both the epithelial injury and changes in inflammatory gene expression (and mediator production) were temporally reproduced. Furthermore, pre-treatment of the fly ash- or vanadium-exposed cells with buthionine sulfoximine (BSO) to deplete glutathione further increased cytotoxicity. Conversely, treatment with the radical scavenger, dimethyl thiourea (DMTU), significantly inhibited these effects in a dose-dependent manner. Overall, the airway epithelial layer appeared to be particularly sensitive to vanadium and its associated oxidative stress. Moreover, the resultant epithelial injury responses appeared to parallel that of gene expression changes (i.e., increased expression of "redox" sensitive IL-6, MIP-2, and iNOS) as well as the increases in inflammatory mediator production/release.

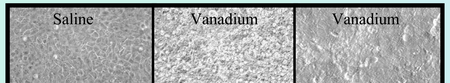
Metal-induced airway epithelial effects



Temporally, vanadium but not nickel or iron (data not shown) sulfate exposure appeared to mediate the majority of the epithelial cytotoxicity induced by the intact fly ash sample. Further, the degree of injury appeared to correlate with the extent of depletion of the ubiquitous intracellular antioxidant, glutathione.

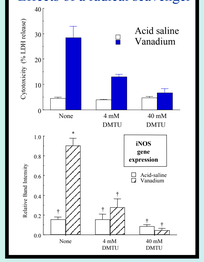


Using RT PCR methods, data indicated that airway epithelial exposure to vanadium for 6 h resulted in induction of IL-6 gene expression. Comparable, somewhat delayed effects were noted for MIP-2 and iNOS gene expression (data not shown).



Similar to the epithelial effects observed during exposure to the intact fly ash sample, over a 6 to 24 h period, vanadium (but not iron or nickel) sulfate exposure resulted in epithelial injury. Compared to saline-exposed cells (A; 40X-phase contrast), vanadium exposure resulted in loss of tight cellular adhesions in the superficial cells and detachment of individual cells or clumps of cells — many of which appear excessively spherical and hyperlucent (i.e., prelytic swelling) (B; 20X-phase contrast). Once these cells were removed via washing, the remaining epithelial layer appeared relatively intact but had areas of "pitting" where individual cells had already detached (C; 40X-HMC).

Effects of a radical scavenger



Associated publications

- Dye JA, Adler KB, Richards JH, Dreher KD. Epithelial injury induced by exposure to residual oil fly ash particles: Role of reactive oxygen species? *Am J Resp Cell Mol Biol*, 1997, 17(5):625-633.
- Martin LD, Krunkosky TM, Dye JA, Fischer BM, Jiang NF, Rochelle LG, Akley NJ, Dreher KL, and Adler KB. The role of reactive oxygen and nitrogen species in the response of airway epithelium to particulates. *Env Health Perspect*, 1997; 105 (Suppl. 5):1301-1307.
- Dye JA, Adler KB, Richards JH, Dreher KL. Role of soluble metals in oil fly ash-induced airway epithelial injury and cytokine gene expression. *Am J Phys 277 (Lung Cell Mol Physiol 21)*, 1999, L498-L510.
- Jiang N, Dreher KL, Dye JA, Li Y, Richards JH, Martin LD, and Adler KB. Residual oil fly ash induces cytotoxicity and mucin secretion by guinea pig epithelial cells via an oxidant-mediated mechanism. *Toxicol Appl Pharmacol* 2000, 163:221-230.

Impact

Improved understanding of potential mechanisms of air pollutant-induced adverse respiratory health effects and establishment of relative toxicity of various PM metal subcomponents.

Conclusions and Implications

- Oxidative stress has been implicated in the pathogenesis of asthma and in the general processes related to aging (Science 2003). Furthermore, metal-mediated generation of reactive oxygen species (ROS) and oxidative stress have been proposed as one of the main mechanisms for emission-source PM toxicity.
- Many transition metals contained in/on PM samples are capable of participating in Fenton-like reactions which could promote excessive radical generation within the lung. Furthermore, a major intracellular source of oxygen radical production is the mitochondrial respiratory chain itself.
- Thus, any disturbance in normal mitochondrial function, owing for example to advanced age, antioxidant deficits, or long-standing inflammation could conceivably enhance electron escape from the electron transport chain. This could result in still greater radical generation within the airways. Moreover, it is known that several transcription factors involved in the regulation of inflammatory processes are activated by intracellular redox changes.
- Through this cascade of events, we *hypothesize* that in susceptible (i.e., asthmatic) individuals, ambient pollutant exposure could indeed culminate in periodic exacerbation of airway inflammation, and thus contribute to perpetuation of asthmatic symptoms and disease.

Future Directions

- We are planning to continue certain aspects of **mechanistic** investigations (i.e., **core efforts**) including investigating potential metal interactions, metal protein-binding, and metal translocation within the lung and airway epithelial layers. We will use rodent genetic/strain-related differences to better understand susceptibility factors underlying the degree of airway injury and inflammation developing after *in vivo* and *in vitro* exposure to PM. Importantly, we will continue to improve upon methods to document cellular redox changes associated with PM and/or oxidant gaseous pollutant exposures.
- Regarding our **"problem driven"** research, we have initiated and plan to continue to study PM from unique sources (including RTP CAPs; see CAPs poster) and to investigate different PM subcomponents (such as metals, see also the poster on PM from the Utah Valley). We will continue to evaluate the effects of metal mixtures and possibly, co-pollutant effects, on the collective toxicity of PM.
- In addition, we are attempting to improve upon PM filter extraction techniques to better establish the toxicity of PM material obtained from archived hi-vol PM filters.

SOLVING AGENCY PROBLEMS